Conserv Genet (2013) 14:783–794
DOI 10.1007/s10592-013-0474-y

RESEARCH ARTICLE

Unravelling landscape variables with multiple approaches to overcome scarce species knowledge: a landscape genetic study of the slow worm

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Received: 22 January 2012 / Accepted: 5 March 2013 / Published online: 24 March 2013

Abstract Landscape genetics was developed to detect landscape elements shaping genetic population structure, including the effects of fragmentation. Multifarious environmental variables can influence gene flow in different ways and expert knowledge is frequently used to construct friction maps. However, the extent of the migration and the movement of single individuals are frequently unknown, especially for non-model species, and friction maps only based on expert knowledge can be misleading. In this study, we used three different methods: isolation by distance (IBD), least-cost modelling and a strip-based approach to disentangle the human implication in the fragmentation process in the slow worm (*Anguis fragilis*), as well as the specific landscape elements shaping the genetic structure in a highly anthropized 16 km^2 area in Switzerland. Friction maps were constructed using expert opinion, but also based on the combination of all possible weightings for all landscape elements. The IBD indicated a significant effect of geographic distance on genetic differentiation. Further approaches demonstrated that highways and railways were the most important elements impeding the gene flow in this area. Surprisingly, we also found that agricultural areas and dense forests seemed to be used as dispersal corridors. These results confirmed that the slow worm has relatively unspecific habitat requirements. Finally, we showed that our models based on expert knowledge performed poorly compared to cautious analysis of each variable. This study demonstrated that landscape genetic analyses should take expert knowledge with caution and exhaustive analyses of each landscape element without a priori knowledge and different methods can be recommended.

Keywords Population genetics · Microsatellite markers · 454 Sequencing · *Anguis fragilis* · Landscape genetics · Least-cost path

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Introduction

Habitat fragmentation, mainly resulting from human activities has an indirect but important impact on the current unprecedented loss of biodiversity (Pimm et al. 1995; Sala et al. 2000; Broquet et al. 2006). Habitat fragmentation splits a species range into "island" patches, with a consequent reduction in population size and migration (gene flow) among these patches (Frankham et al. 2010). Small population sizes and lack of individual exchange between isolated demes can further decrease genetic diversity and consequently impact the survival rate and evolutionary potential in all patches, leading to an increased likelihood of patch extinction (Frankham 2005).

Gene flow between land-dwelling populations is significantly affected by the landscape matrix and land-use resistance to migration (Moilanen and Hanski 2001). Statistical methods have been developed to evaluate the impact of the landscape on the spatial genetic structure of populations. These methods aim to detect the landscape elements influencing the gene flow between demes, and to quantify their impact on migration. In most cases, Isolation by Distance (IBD, Wright 1943) explains only a small proportion of the observed genetic differentiation and, to disentangle the effect of geographical distance from that of the landscape matrix, further landscape genetics methods were developed (Holderegger and Wagner 2006). A commonly used approach is based on least-cost paths between demes. In this approach, friction maps are computed, where each raster cell is given a specific value representing the degree of resistance to migration for a specific species. According to these maps the lowest resistance path between each pair of populations is calculated (Adriaensen et al. 2003; Coulon et al. 2004; Ray 2005; Rouget et al. 2006; Schwartz et al. 2009). Friction maps are typically based on expert knowledge, using a priori weight values for each landscape variable, raising the issues of translating and weighting these variables. Consequently, the resulting friction maps can be strongly biased. An alternative methodology, the strip based method, was recently proposed by Emaresi and collaborators (Emaresi et al. 2011), in order to avoid a priori assumptions. In this approach, the frequency of each landscape variable is assessed in straight-line pairwise strips of varying width.

In this study, landscape genetic approaches were used to understand the landscape elements impeding or favouring gene flow between populations of the little-studied slow worm, *Anguis fragilis*. This reptile, like other members of the family *Anguidae,* is an elongated legless lizard native to Eurasia. It can be found in a large variety of natural habitats like shrub vegetation and forest edges, as well as human influenced areas such as gardens or parks (Völkl and Alfermann 2007). Although very secretive, this species is one of the most widespread reptiles in Europe. However, studies focusing on this species remain rare and most information has been gathered during monitoring experiments (Völkl and Alfermann 2007). Knowledge of slow worm dispersal is scarce; Stumpel (1985, cited in Völkl and Alfermann 2007) observed that slow worms stayed mainly at the same location. One, far-moving, individual travelled 80 m in 7 days and a maximum distance of 130 m in 2 years. In another monitoring experiment, Plattenberg (1999, cited in Völkl and Alfermann 2007) showed an average distance between recaptures of 12–16 m. In Europe, the threat to this species remains low and, for instance in Switzerland, the slow worm threat status is considered to be of "least concern" by the Swiss Reptile Red List (Monney and Meyer 2005). These authors, however, pointed out a lack of knowledge of the spatial distribution of this species in Switzerland (as in most other European countries). Moreover, they suggested that slow worm occurrence appears to be declining in the Swiss Plateau and in the lower part of the Alpine valleys according to local inventories (Monney and Meyer 2005).

To our knowledge, no population genetic studies of slow worms have been conducted to date. Therefore, we developed nine novel microsatellite markers for this species, taking advantage of next-generation sequencing. We assessed the genetic structure in the slow worm at a small scale (16 km^2) and investigated the possible impact of landscape elements on gene flow. We tested for isolation-by-distance as a null model followed by investigation of two alternative approaches (least-cost path modelling and a strip-based method) that explicitly implement landscape elements. In order to test the a priori knowledge of the species behaviour and naive weighting of each landscape elements, different scenarios were conducted and compared. Finally, with respect to the advantages and drawbacks of each method, we assessed the effects of the different landscape components and the extent to which they represent a dispersal barrier.

Materials and methods

Sampling

The sampling was conducted between Lausanne and Geneva (Switzerland), in the region "La Côte" (Fig. 1). The selected area (16 km^2) represents a typical, highly anthropized, fragmented landscape dominated by agriculture (vineyards, cereal crops and pastures), villages and a large dense forest, as well as small forest patches. In addition, linear elements such as rivers, railroads, a highway and numerous secondary roads are present.

Slow worms were collected from 15 sites (out of 32 tested) using black tar plates (used as a cover by the reptiles). Saliva samples were taken from 118 animals using buccal swabs (Miller 2006; Beebee 2008). All captured animals were measured, weighed and photographically identified in order to avoid repeated sampling of the same individual.

Microsatellite marker development

Since no highly variable nuclear markers were available for the slow worm, we developed specific microsatellite markers for further population genetic analyses. The method used was identical to that of Metzger et al. (2011). Briefly, random reads of the complete genome of a single slow worm were

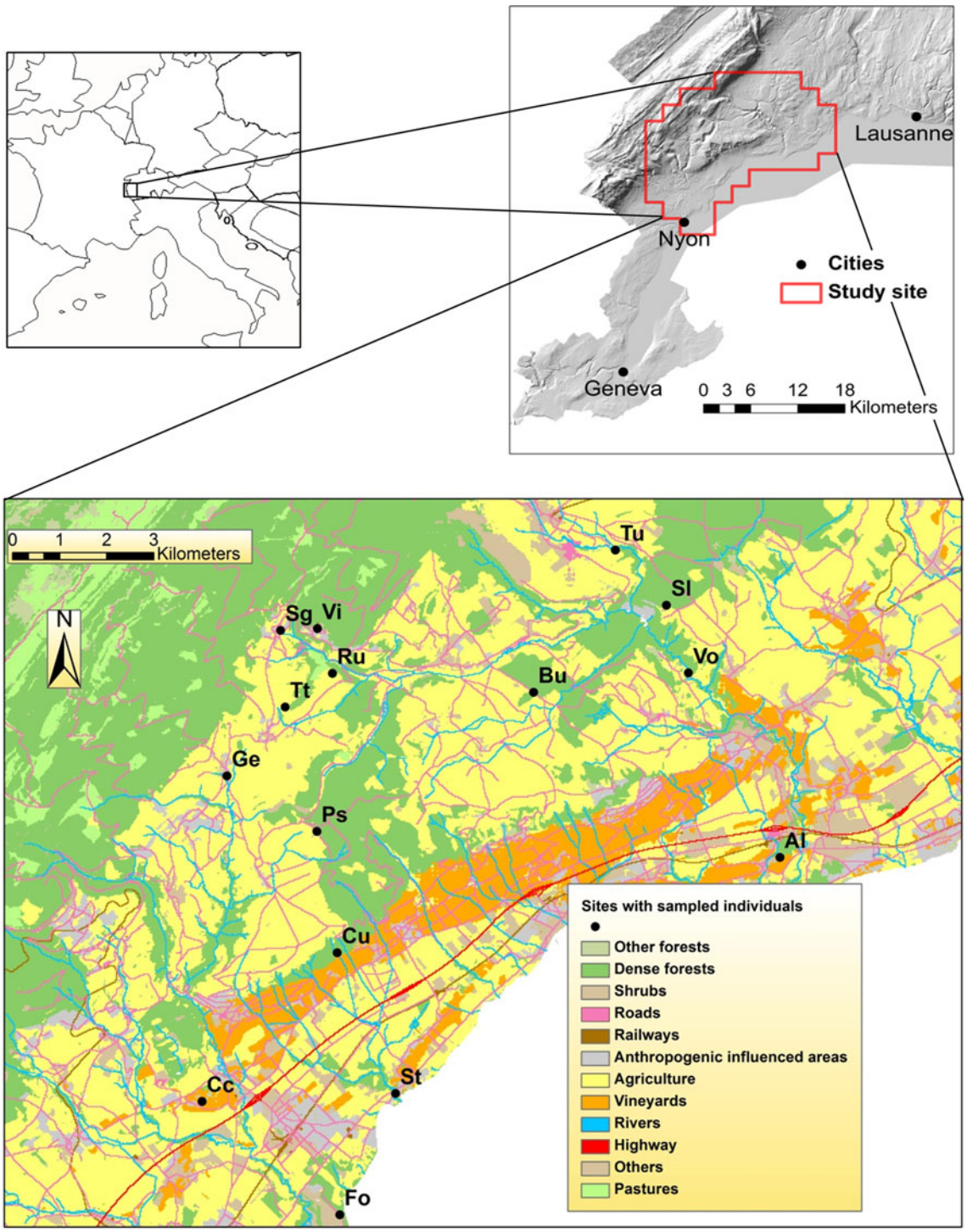

Fig. 1 Repartitioning of the sampled sites in Switzerland (between Lausanne and Geneva) with the landscape elements used for the landscape genetic analysis. The *black dots* represent sites where slow worms were sampled. Abbreviations correspond to those in Table 3. (Color figure online)

obtained using 454 shotgun sequencing. Reads were screened for potential microsatellites using MSATCOMMANDER v0.8.2 (Faircloth 2008) and a final selection was made by eye based on the length and homogeneity of their repetitions with SPOTLIGHT (Mac OS X 10.6). Furthermore, PCR amplifications were conducted on a Mastercycler Gradient (Eppendorf, Schönenbuch/Basel, Switzerland) with specific newly designed primers and tested with different annealing temperatures and $MgCl_2$ concentrations. PCR products were then assessed by agarose

gel (1 %) electrophoresis. Polymorphism was examined using a multicapillary electrophoresis system (QIAxcel System; QIAGEN, Hombrechtikon, Switzerland) on 11 sampled animals from different populations of the study area.

DNA extraction and population genetic analyses

Genomic DNA was extracted from the buccal swabs with a Qiagen DNeasy Kit (QIAGEN, Hombrechtikon, Switzerland) following the manufacturer's protocol except regarding the following points: (i) incubation was performed overnight at 56 °C; (ii) the swab was introduced into the column during step 3 and a supplementary centrifugation step after removing the buccal swab was added. PCR amplifications were conducted for all loci and all individuals following the PCR conditions previously set up (Table 1) using fluorescently-labelled forward primers (total volume of 10 µl containing 3–4 µl template DNA, 1.75 µM of each primer, 0.2 mM dNTP, 2 mg/ml Qsol, $MgCl_2$ according to Table 1 and 0.5 U TAQ). The PCR products were analysed on an AB3130xl sequencer (Applied Biosystems, Carlsbad, California). Allele length was scored by visually identifying the microsatellite peaks with PEAK SCANNER™ software v1.0 (Applied Biosystems, Carlsbad, California).

First null alleles, large allelic dropout and stutter errors for each site and locus were tested using MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004). Locus pairs were tested for linkage disequilibrium using FSTAT v2.9.3.2 (Goudet 1995). Population differentiation (F_{ST}), inbreeding coefficient (F_{IS}, Weir and Cockerham 1984), expected (H_e) and observed (H_o) heterozygosity, allelic richness (A) and deviation from Hardy–Weinberg (HW) equilibrium were assessed using FSTAT. Significances levels on pairwise genetic differentiation were evaluated based on 1,000 simulations. Due to high differences in sampling sizes at the different sites, we also compared all mean F_{ST} for each site in order to test for the occurrence of possible outliers.

Population genetic clustering was analysed with STRUCTURE v2.3.3 (Pritchard et al. 2000) using the admixture model (100,000 iterations, with a burn-in of 20,000 iterations). We used both the highest *ln* values and the approach suggested by Evanno and collaborators (Evanno et al. 2005) to evaluate the most likely cluster number.

Landscape genetics

A raster map with a cell size fixed to 25 m was obtained by combining the Swiss national topographical map (1:25,000

Table 1 Characteristics of the nine microsatellite loci of *Anguis fragilis* tested on 118 individuals, with the primer sequences, annealing temperatures, $MgCl_2$ concentrations and levels of microsatellite variability (the number of alleles, observed [H_o] and expected [H_s] heterozygosity and F_{IS} values was based on 118 individuals) estimated with FSTAT 2.9.3 (Goudet 1995)

Locus	Primer sequence	Repeated motif	Annealing temperature	$MgCl_2$ concentration (mM)	No. alleles	H_0	H_s	F_{IS}
Afl9	CAG TGA TTG TGT GGT GTT TAT CTC TCT AGG AGT CTG AGT TTC GGC	$(CAA)_{13}$	55 °C	3	4	0.375	0.373	0.034
Af22	CAG ATT GCT GAC TGG GAC C GTG ATC TCT GGG AAG TGC CTC	$(TTAT)_8$	55 °C	3	5	0.66	0.552	−0.224
Af24	GCT AGG TAG CGT TCT CC GGGACAGAGCACTTTGTGTG	$(ATT)_8$	50 °C	1.5	3	0.375	0.395	−0.005
Af34	CCA CAC TCT ACA TGG ACT GC CAC TCT GGA TTA AGT CAA GG	$(GT)_{11}$	55 °C	3	7	0.512	0.649	0.157
Af37	GCA TAC ATC AAG TAA CC TCC CTT GTA AAC TGC CCT G	$(GAT)_{14}$	55 °C	3	3	0.244	0.222	−0.103
Af38	AGA CAG ATA TTT CCC TTG TCA ACC CCA TTG TCG CAG CCA GGC AC	$(ATT)_{12}$	50 °C	1.5	5	0.352	0.352	−0.033
Af44	GCC AGG GAA AAC ATA GAT GC CTG TAA ACT GCC GAG TGA G	$(TCTT)_7$	60 °C	3	4	0.252	0.265	0.014
Af47	GGT GGT AGA ATG AAC TG CTG GAT GTT GGT GTA GAT G	$(ACC)_{11}$	52 °C	3	4	0.476	0.452	−0.047
Af50	GTC TTG TAG CCC TTT TCC GTC TGT GAA CTT AGT GTC CG	$(CA)_{18}$	52 °C	1.5	5	0.642	0.601	0.601

scale, Swisstopo) with a land-use classification from photo-interpretation (Lehmann et al. 2000). The result was a precise and detailed map with a resolution of 25 m integrating 61 land-use categories, which can be used for studies at a regional scale.

Isolation by distance (IBD)

The hypothesis of IBD was tested by comparing corrected genetic differentiation ($F_{ST}/[1-F_{ST}]$) with the transformed geographical distance (ln[dist]; Rousset 1997). Significance of the correlation was tested using a Mantel test (10,000 permutations) performed with R (R Development Core Team 2011) using the NCF package (Ottar N. Bjornstad, ncf: spatial nonparametric covariance functions, R package version 1.1-3, 2009).

Least-cost modelling

Least-cost modelling is a widely used method in ecology (Broquet et al. 2006; Epps et al. 2007; Schwartz et al. 2009). A friction map based on the attribution of specific weight to each different environmental parameter was created. The given value attributed to each category represents the degree of resistance of the specific landscape type and used to compute the least-cost path. Due to the limited and contradictory knowledge about dispersal and habitat uses by the slow worm, different scenarios were tested (see Fig. 2):

Scenario 1—strong effect of dense forest, highways and rivers For scenario 1 we selected 12 land uses (dense forests, other forests, shrub and bush vegetation, roads, railways, anthropogenic-influenced areas, agriculture, vineyards, rivers, highway, pastures and other land uses) that could harbour slow worm populations or impede the dispersal of this species. The first hypothesis relied on a higher fragmenting effect of dense forests, highways and rivers (Table 2). Consequently, a higher degree of resistance *C* compared to the degree of resistance *c* given to all other landscape elements was allocated. We tested four different *C*:*c* ratios (4:1, 10:1, 30:1 and 40:1) to evaluate the sensitivity of this model.

Scenario 2—a priori knowledge of the species behaviour The second scenario was based on the hypothesis that primary and secondary habitats favoured dispersal. Each of the initial 61 categories of the raster map were reclassified into five types (1. primary habitat; 2. partly suitable primary habitat; 3. secondary habitat; 4. partly suitable secondary habitat and 5. Non-habitat) according to the preferred habitat of the slow worm as described by Völkl and Alfermann (2007). A friction map weighting the five categories in different ways was calculated with ascending or equal weights for category one to four, the fifth category (non-habitat) always having the highest friction weight.

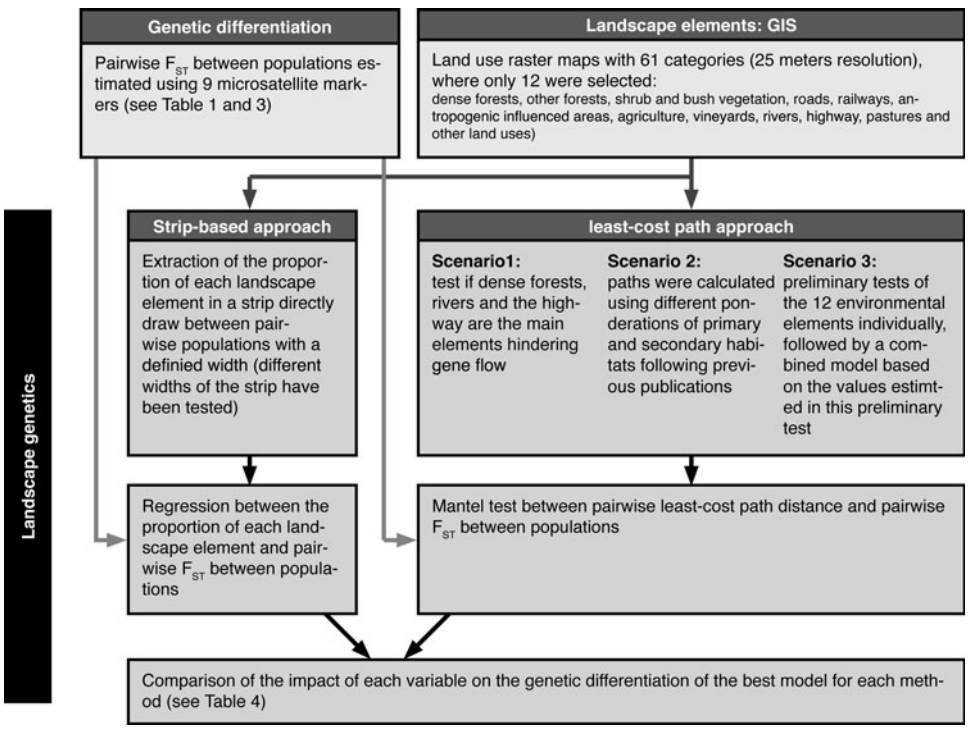

Fig. 2 Summary of the different landscape genetic approaches used in this study: the strip-based approach following that of Emaresi et al. (2011) on the *left* and the least-cost path method on the *right*

Table 2 The three least-cost path modelling scenarios: the 12 most prominent environment categories were retained for scenarios 1 and 3

	Scenario 1 Costs	Scenario 2	Scenario 3 Costs
Other forests	1	All 61 categories were classified into five categories according to their suitability for slow worms (following Völkl and Alfermann 2007). They were combined with ascending weights or equal weight for category one to four (see "Materials and methods" section for more details)	2/4/8/10/15/20/30/40/60/1
Dense forests	4/10/30/40		2/4/8/10/15/20/30/40/60/1
Shrub and bush vegetation	1		2/4/8/10/15/20/30/40/60/1
Roads	1		2/4/8/10/15/20/30/40/60/1
Railways	1		2/4/8/10/15/20/30/40/60/1
Anthropogenic-influenced areas	1		2/4/8/10/15/20/30/40/60/1
Agriculture	1		2/4/8/10/15/20/30/40/60/1
Vineyards	1		2/4/8/10/15/20/30/40/60/1
Rivers	4/10/30/40		2/4/8/10/15/20/30/40/60/1
Highway	4/10/30/40		2/4/8/10/15/20/30/40/60/1
Other land uses	1		2/4/8/10/15/20/30/40/60/1
Pastures	1		2/4/8/10/15/20/30/40/60/1

Based on the hypothesis that only dense forests, rivers and the highway have an impact on the dispersion ability of slow worms, the cost of only these three variables were considered, all the others having a cost of 1 (i.e., putatively no barrier to dispersal) in the first scenario. In scenario 2, all 61 environmental variables were taken into account and classified into their putative habitat quality (see "Materials and methods" section for more details). In scenario 3, the 12 selected variables were first tested one by one with various costs and then the best association accounting for the previously assessed costs were inferred

Scenario 3—naïve scenario For scenario 3 we selected 12 land uses (dense forests, other forests, shrub and bush vegetation, roads, railways, anthropogenic-influenced areas, agriculture, vineyards, rivers, highway, pastures and other land uses) that could harbour slow worm populations or impede the dispersal of this species. In this scenario we wanted to overcome the lack of knowledge about habitat preferences, the uncertainty in weighting the different variables and disentangle the effect of each land-use. We first analysed the impact of different costs (2, 4, 8, 10, 15, 20, 30, 40, 60 and 80) for each variable separately, all other land uses having a cost of 1. A lower weight (1 for the focus variable and costs varying between 2 and 60 for all other variables) was also tested as a control. The best model for each variable was selected based on the highest significant correlation coefficient.

Further, all variables were pooled into a single model to test for their combined effect with respect to the assessed weights obtained by the former one-by-one tests (Table 2) and the model sensitivity was tested.

For each model, pairwise least-cost paths were calculated using the extension PATHMATRIX (Ray 2005) implemented in ARCVIEW 3 (Environmental Science Research Institute, Redlands, USA) to compute matrices of effective geographical distances (EGD) among the 13 sample sites. To select the best model and test the sensitivity of each simulation, Mantel tests (Mantel 1967) were conducted between corrected genetic distances (following Rousset 1997) and the logarithm of EGDs using the software MANTELN (Ray and Excoffier 2003) with 10,000 permutations.

For the best model, we calculated partial correlations controlling for Euclidean distances with a partial Mantel test included in the R package NCF in order to disentangle the part of the model explained by landscape elements alone corrected by the effect of distance. Both least-cost distances (LCD, i.e., the accumulative cost along the least-cost path) and along least-cost path distances (APD, i.e., the length in metres of the least-cost path) were calculated for each model.

Strip-based approach

The strip-based approach identifies the main landscape elements influencing gene flow in straight-line strips, without any a priori assumptions. This method was developed by Emaresi et al. (2011) and its implementation in the software FRICTIONNATOR (http://www2.unil.ch/biomapper/frictionnator/frictionnator.html) allows the extraction of the land use densities in each strip. Multiple generalised linear regressions (Gaussian) on independent variables were conducted to (i) select the best strip width and (ii) analyse the effect of each landscape variables (see above) on dispersal. The first step consisted of testing the impact of Euclidean distance on genetic differentiation [glm (Fst ~ "Euclidean distances")], each of the 12 chosen land uses (the same as that of scenario 1 and 3 of the least-cost modelling) were added to this null model one by one (e.g., [glm (Fst ~ "Euclidean distances" + "Land use 1…12"]).

Aikaike's information criterion (AIC) was used to assess the relative likelihood of each model. The correlation coefficient (r^2) and the slope of the regression were calculated to evaluate the effect of each variable and whether this variable impeded (+) or facilitated (−) gene flow (see Fig. 2).

Different strip widths were tested (75, 125, 275 and 525 m, as well as a width:length ratio of 1:1, 1:3 and 1:7). To select the best strip width model, mean Aikaike's information criterion (AIC) was computed. To investigate the specific contribution of each variable, weighted AICs were calculated.

F_{ST} values were previously transformed and successfully tested for normality with the Shapiro–Wilk test ($p < 0.05$). All statistical analyses were performed with R.

Comparison of the two approaches

Since the two approaches rely on different mathematical assumptions and methods, we focused the comparisons only on the proportional contribution of each environmental variable acting as a barrier to gene flow. In the least-cost path method, the EGDs were highly correlated with the geographical distances per se, but in the strip based method, the geographical distances were included in each regression as a co-variable. Consequently the pairwise Euclidean distances were included in the landscape variables in each method but at different stage of the analysis. To perform the comparison, we assessed the relative costs of each variable in the least-cost path approach and the relative R^2 in the strip-based approach. However, due to the separate bivariate analysis in the strip-based approach, the weighted AICs had to be taken into account too when comparing variables inside a model.

Results

Microsatellite marker development

Out of the 18'190 reads produced by the 454 sequencing, MSATCOMMANDER found 1'987 microsatellite loci. Based on their homogeneity, only 33 sequences were selected and specific primers designed. After PCR optimisation with different alignment temperatures and $MgCl_2$ concentrations, successful amplifications were obtained for 27 loci (82 %). However, only 13 microsatellite markers (39 % of the sequences for which a primer was designed) demonstrated polymorphisms for 11 individuals from different populations when analysed by the QIAxcel system.

Population genetics analyses

Out of the 13 polymorphic microsatellite markers tested on the capillary agarose system, only ten loci could be successfully genotyped by ABI sequencing. One locus (Af19) showed a slight excess of homozygotes in a single population (St), suggesting the possible occurrence of null alleles. Due to its occurrence in a single population, we decided to keep this locus for further analyses. Moreover, the locus Af46 showed significant linkage disequilibrium with loci Af47 and Af50 (adjusted $p = 0.001$). Therefore, this locus was excluded from further analyses and genotyping was conducted with the nine remaining microsatellite loci (Af19, Af22, Af24, Af34, Af37, Af38, Af44, Af47 and Af50) showing no significant deviation from Hardy–Weinberg equilibrium.

The number of alleles, expected and observed heterozygosity and F_{IS} for each locus are provided in Table 3. The number of alleles per locus ranged from 3 to 7, with the expected heterozygosity ranging from 0.244 to 0.649. The F_{ST} values ranged between 0.03 and 0.17, with only 19 % of all comparisons significant. Significant pairwise F_{ST} values ranged between 0.047 and 0.174, and only three values exceeded 0.1 (Ge-Bu: 0.111, Sg-Bu: 0.175 and Al-St: 0.175). No significant differences of mean F_{ST} values were observed (Kruskal–Wallis rank sum test: $p > 0.05$), suggesting that the differences were not imputable to the sample size.

The global F_{IS} value was low (−0.035), with only two sites (Cc and Cu) showing a higher level of inbreeding (respectively 0.2 and 0.123); however, no F_{IS} values were significant.

The STRUCTURE simulations suggested the occurrence of a single cluster, indicating that all individuals belonged to a single population.

Landscape genetics

Isolation by distance

A significant relationship (Mantel's $r = 0.250$, $p = 0.022$) was found between corrected genetic distance and logarithmic geographical distance for 13 sites (F_{ST} values were only calculated for populations with $N > 3$ individuals).

Least-cost modelling

For scenario 1–3, for each different friction map tested except one, the APD showed higher significant correlations than the LCD and this difference was significant testing all correlation in the three scenarios (p value <0.05). Therefore, only APDs are presented here and used for the landscape analyses.

Table 3 Detail information of each sampling site with the number of individuals analysed, F_{IS} and mean F_{ST} values

SITE	Number of samples	F_{IS}	Mean F_{ST}
Al	3	−0.300	0.140
Bu	7	−0.175	0.099
Cc	3	0.200	0.082
Cu	9	0.123	0.043
Fo	3	0	0.104
Ge	16	−0.086	0.073
Ps	4	0.053	0.033
Ru	8	0.027	0.038
Sg	10	−0.047	0.079
Sl	27	−0.011	0.060
St	23	−0.001	0.083
Tt	1	NA	NA
Tu	3	0	0.080
Vi	3	−0.021	0.092
Vo	1	NA	NA
Mean	8	−0.035	0.077

In the first scenario, a low weight of 4 allocated to dense forests, highway and rivers gave the best correlation (Mantel's $r = 0.262$, $p = 0.015$). Allocating higher costs to these three landscape types gave lower correlations compared to IBD.

In the second scenario, the friction maps with a weight of 60 for the « non-habitat » type performed poorly; some simulations even resulted in non-significant relationships between genetic distance and APD. Weighting the five habitat types in ascending order produced slightly better results, the best model having the following weight—from type 1 to type 5: 3, 4, 10, 11 and 20; Mantel's $r = 0.256$, $p = 0.020$). In this model the differences between habitats and non-habitats were low.

In the third scenario, each variable was first tested separately with different weights and we selected the weight with the highest significant correlation for each variable (details not shown). When correlation values were identical, we selected the model with the lowest p value. About 20 new models were compiled with respect to the proportionality of costs assessed before. The best scenario (Mantel's $r = 0.286$, $p = 0.013$) was performed with a high cost (75) for roads, railways and vineyards, an intermediate cost (40) for rivers and highway and a very low cost (1 or 2) for the other variables (dense forests, other forests, shrub and bush vegetation, anthropogenic-influenced areas, agriculture, pastures and other land uses).

Comparing all scenarios of the least-cost path methods, the impact of each landscape parameter was best explained by Scenario 3. Conversely, Scenario 2, based on the habitat knowledge, generated the worst results.

Finally, a partial Mantel test allowed disentangling the effect of effective geographical distance without the Euclidean distance in the best model. Partial Mantel tests showed that the genetic distances correlated better with the along least-cost path distances (Mantel's $r = 0.284$, $p = 0.016$) than with the Euclidean distances alone (Mantel's $r = 0.248$, $p = 0.027$). However, parsing out the Euclidean distances from the APD was essential to assess the part of the correlation explained by the landscape elements alone. This correlation was positive (Mantel's $r = 0.218$) and marginally significant ($p = 0.057$).

Strip-based approach

We first tested the different strip widths and the best model (lowest AIC score) was obtained with a fixed width of 525 m (AIC = 32.05). With this width, 3 variables provided a better explanation of genetic differentiation than the Euclidean distance alone (wAIC = 0.048): railway (wAIC = 0.313), highway (wAIC = 0.059) and other land types (wAIC = 0.058). These three variables accounted altogether for 41.5 % (16.3, 12.6 and 12.6 %, respectively) of the explained variance of the model and all had a negative impact on gene flow (positive sign of the regression S_i). Since railways and the highway are parallel and very close to each other in the study area, it was relevant to sum up both variables to demonstrate a strong, negative influence of these structures (about 29 %) in the model. Only two variables had a positive effect on gene flow: agriculture and dense forests, accounting for about 20 % of the variance.

Comparison of the landscape genetics results

When comparing the three landscape genetic models, the IBD model performed poorly. Indeed, partialling out of the Euclidean distance for the least-cost path method, as well as the low wAIC values of the Euclidean distance variable alone, suggested that the distance explained only a small part of the genetic differentiation.

The effect of each element impeding gene flow is presented in Table 4. With both methods, railways and the highway showed the highest impact. Three other elements ("other land use", "anthropogenic-influenced areas" and "other forests") showed a negative effect using the strip-based approach, but no negative effect in the least-cost path modelling. By contrast, "roads" and "vineyards" showed a high negative effect in the least-cost path modelling, but only a marginal effect in the strip-based approach. "Rivers" had a relatively low effect in both analyses. Finally,

Table 4 Comparison of the environmental elements negatively impeding gene flow in the least-cost path and strip-based approaches

Variable	Least-cost modelling	Strip-based approach
Railway	24 %	20 %
Highway	13 %	15 %
Other land use	No	15 %
Anthropogenic-influenced areas	No	13 %
Agriculture	No	No
Dense forests	<1 %	No
Other forests	No	10 %
Roads	24 %	10 %
Rivers	13 %	9 %
Vineyards	24 %	8 %

Environmental elements favouring gene flow were not taken into account when assessing their role in fragmentation in the two approaches. They are consequently noted with a "No"

"agriculture" and "dense forests" showed a very low effect on gene flow in both approaches.

Discussion

Habitat fragmentation represents a serious threat to the long-term viability of animal populations (Fahrig 2003). Numerous studies have demonstrated the negative effects on genetic diversity and demography, resulting in the loss of connectivity between habitat patches and population size reduction (Cushman 2006; Walker et al. 2008). A previous publication (Monney and Meyer 2005) suggested shrinking of slow worm populations in the Swiss Plateau and in the lower part of the Swiss Alpine valleys. In this study, we show that habitat fragmentation, in particular human-induced fragmentation, as well as isolation-by-distance affects the genetic differentiation among slow worm demes. However, the overall low genetic differentiation ($F_{ST} = 0.077$) and the lack of distinct clusters determined by STRUCTURE suggest that gene flow between the different sites still occurs in some extent, even though some landscape elements or geographical distance might have a negative effect on it. Consequently, the dispersal ability of the species seems to be sufficient in avoiding strong isolation in human-impacted habitats, at least until now in our study area.

The absence of inbreeding and substructure in the study area (16 km^2) supports the hypothesis that the dispersal capacity of the slow worm has been strongly underestimated and that some individuals migrate over long distances, allowing gene exchange (Völkl and Alfermann 2007). However, assessing the dispersal behaviour of single animals (e.g., using telemetry or long-term capture-recapture studies) will be necessary to fully understand the population dynamics and dispersal abilities of the slow worm, as well as improving the conservation of this species.

Landscape genetics

As suggested by Excoffier and Heckel (2006), we used several approaches to evaluate the impact of the different landscape elements on shaping the genetic structure. First, a significant IBD effect was detected. Regarding the scale of the sample region and the dispersal abilities of lizards, especially slow worms (Völkl and Alfermann 2007), it was not surprising that more distant populations showed higher genetic differentiation.

Nevertheless, the limited effect of IBD (Mantel's $r = 0.250$) per se and Euclidean distances included in the models showed that geographical distance is only one factor responsible for the genetic fragmentation of slow worm populations. The least-cost path and strip-based approaches demonstrated a joint effect (37 and 35 %, respectively) of the highway and the railroads crossing the study site. In addition, these barriers were strongly detected by both methods to have a higher impact on gene flow than geographical distance (according to wAIC comparisons). Vineyards were also found to be a barrier to gene flow by both methods, although to a different extent. Since this landscape element is parallel to the highway and railway in the studied area, disentangling the effects of these elements would need further detailed studies with additional sites. Dense forests and agricultural areas seem to be used as dispersal corridors, although other habitats have been judged more favourable for the species. The study area is strongly influenced by human activities and our results suggest that slow worms disperse using natural or artificial refuges to hide, warm up and feed in anthropized areas (i.e. agricultural areas) and forests. These results also suggest that slow worms occasionally cross roads with little traffic. Rivers, in the studied region, mostly occur in natural areas with bridges allowing migration, thus, explaining their limited impact on fragmentation. According to the assessed impact on gene flow of different landscape elements, populations will probably become increasingly differentiated on each side of the railway and motorway in the future due to only few possibilities to cross these elements.

For the conservation of the species, we showed that slow worms are able to disperse across this highly anthropized region, coping with human-induced fragmentation except

for the highway. This will probably allow slow worm populations to persist in such anthropized habitat. Even if there is no urgent need to adjust the current protection of the slow worm, we can advocate that improving connection between both side of the highway would reduce the threats and damaging consequences of fragmentation in the study area.

Comparisons of the different approaches

Generally speaking, the least-cost path analyses showed that using previous expert knowledge to choose and weight the respective landscape elements for the creation of the friction map could lead to misleading results. Actually, the second scenario, based on habitat preferences described in Völkl and Alfermann (2007) showed the worst results. First, the habitat where the species is mainly found does not necessarily reflect migration corridors used by the species. In addition, habitat knowledge based on presence during monitoring observations can be scarce and can reflect only partially the habitat use. Further, the arbitrary initial weighting of variables is a sensitive step, and different weightings should be cautiously tested. The naïve scenario, even time consuming, has the advantage of evaluating the relative impact of each landscape element separately. It allowed a relevant combined model that performed the most efficiently. The respective weight of each land use type provided indirect information about the ecology of the species, i.e. the migration corridors.

Beside the difficulty of assessing biologically meaningful landscape variables, this study also shows the need for different methods to unravel their relative importance. Least-cost path and strip-based methods both similarly identified the railway and highways as impediments gene flow; for other environmental variables, the results of the two methods were sometimes contradictory. For example, roads and rivers showed a lower negative effect on population differentiation in the strip-based approach. In this method, the presence of a strong but narrow barrier, perpendicular to the migration path, represents only a very limited proportion of the whole habitat within the strip between both populations. This very low frequency can lead to an underdetection of such a strong obstacle (e.g. roads). On the contrary, the least-cost path methodology is highly sensitive to the occurrence of such an element between two demes. The least-cost methodology therefore probably represents a better approach to detect linear barriers and more generally heterogeneities and spatial arrangements of landscape elements, since EGD calculated according to a friction map is more realistic than straight strips (see Fig. 3 in "Appendix").

As highlighted by Balkenhol et al. (2009) and others, there is a real need to improve statistical methods for landscape genetics. For instance, Legendre and Fortin (2010) recommended the use of multiple regressions instead of Mantel test when investigating environmental and spatial response variables. Therefore, the strip-based approach, based on generalized multiple regression, allows the analyses the effect of each variable separately (controlling for distance) overcoming also the drawbacks of the Mantel test (underestimation of type I error). However, due to the lack of consensus about the most adapted methods, we suggest to use several methodologies and compare the results in order to get robust inferences.

In summary, the development of new and specific microsatellite markers for slow worms revealed indirectly a high dispersal capacity. For this species, simple models based on expert knowledge performed poorly, since they did not catch the maximum complexity of barriers to gene flow and the use of suboptimal habitats for emigration. On the contrary, a naïve model including a comprehensive analysis of each variable separately allows accounting for the influence of each variable using the maximum of information provided by the dataset. This cautious variable selection and their relative weights led to a more accurate detection of the landscape elements influencing gene flow (such as the highway and the railway for the slow worm). Further, combining the results of several approaches helped to overcome methodological issues (e.g. underdetection of linear elements) and discard misleading or confirm the strong effect of one variable (e.g. roads or the highway in this study). More generally speaking, we strongly suggest to take previous knowledge with care when constructing friction maps. The habitat mainly used by a species does not necessary reflect the possible dispersal corridor of this species. Consequently, friction maps should not only be constructed using the current species knowledge, but also in a naïve way, evaluating all landscape elements independently. Even if this approach is more time consuming, it will avoid incorrect interpretation and can also improve the knowledge on the dispersal abilities of the examined species.

Acknowledgments Samples were taken with the permission of the Conservation de la Faune du Canton de Vaud (Switzerland). Field work was made possible by the numerous owners allowing the installation of plates on their lot. We acknowledge Matthieu Raemy and Hans-Peter Rusterholz for their help in the laboratory. In addition, discussions with Jean-Claude Monney greatly improved the manuscript.

Appendix

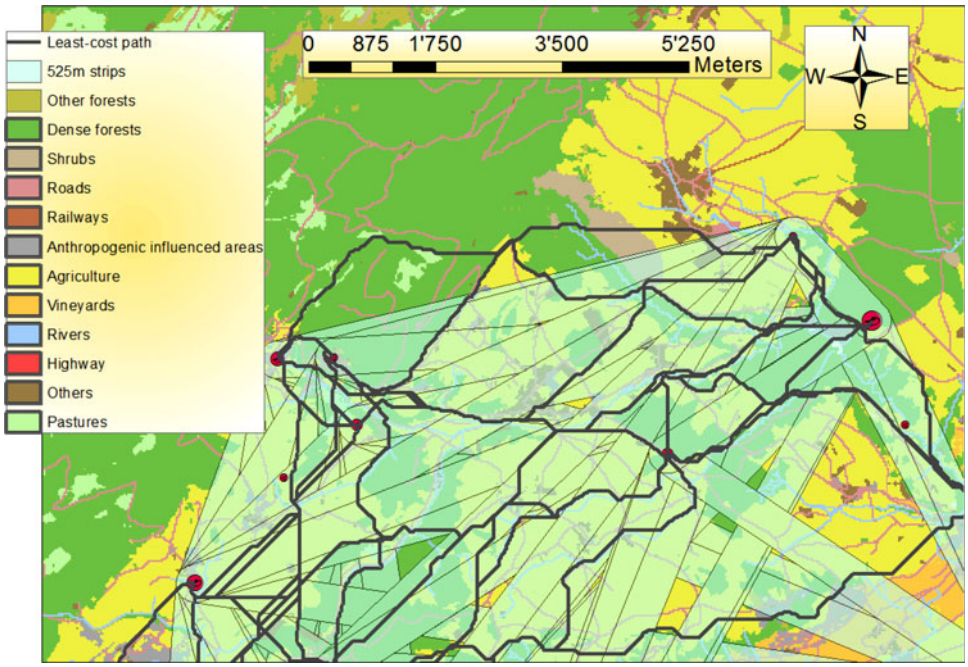

Fig. 3 A part (northern part) of the studied area with pairwise least-cost paths (*black lines*) of the best model and the pairwise straight 525-m wide strips of the best model (*light green stripes*). (Color figure online)

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